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Kidney International, Vol. 32 (1987), pp. 31–38

Procoagulant activity in kidneys of normal and bacterial lipopolysaccharide–treated rabbits

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Procoagulant activity in kidneys of normal and bacterial lipopolysaccharide–treated rabbits. Fibrin formation in the kidney is frequently associated with clinically–significant renal dysfunction. We therefore measured and characterized the procoagulant activity (PCA) which is present in normal kidneys and in kidneys of rabbits with the Shwartzman phenomenon induced by two injections of bacterial lipopolysaccharide (LPS; E. coli LPS 055:B5,25 μg/kg and 50 μg/kg administered 24 hrs apart with rabbits sacrificed 12 hrs after the second injection). PCA was measured in sonicated tissue by one–stage coagulation assay. In normal kidneys the amounts of PCA in the inner medulla, outer medulla and inner cortex were 18.2 ± 3.2, 44.1 ± 3.8 and 78.5 ± 5.7 percent, respectively, of that in the outer cortex (N = 31). Glomeruli (purified by the iron oxide magnetic method to greater than 95 percent homogeneity) contained 21.6 ± 8.8 arbitrary units/μg protein compared with tubular fragments which contained 13.9 ± 2.6 U/μg protein (N = 9). In LPS-treated rabbits PCA (in units/μg) increased in outer cortex from 33.7 ± 3.9 (control) to 73.4 ± 10.4 (LPS, $P < 0.01$), in inner cortex from 26.7 ± 2.9 (control) to 83.3 ± 17 (LPS, $P < 0.02$), in outer medulla from 12.9 ± 2.4 (control) to 54.5 ± 16.5 (LPS, $P < 0.05$), and in inner medulla from 12.2 ± 2.4 (control) to 32.1 ± 4.9 (LPS, $P < 0.01$). Glomerular PCA increased from 21.6 ± 8.8 (control) to 88.8 ± 20.7 (LPS) units/μg (P = 0.01), while tubular fragment preparation PCA increased from 13.9 ± 2.6 (control) to 44.6 ± 12.7 (LPS) U/μg (P = 0.02) (N = 9 per group). PCA was thromboplastin–like as judged by clotting assay using human deficient plasmas. Fibrin and monocytes were present within capillaries of glomeruli of LPS-treated rabbits. In contrast, isolated glomeruli incubated with LPS for six or 21 hours showed no increase in PCA. We conclude that most of the PCA in normal kidneys is in the cortex. The normal renal medulla contains only small amounts of PCA. PCA is distributed to both glomeruli and tubules. Following LPS injection PCA increased in all structures examined, particularly in the glomeruli where extensive fibrin formation had occurred. This increase in glomerular PCA may have been related to the influx of mononuclear cells.

Intraglomerular fibrin deposits are a frequent finding associated with many forms of renal dysfunction including sepsis, toxemia and peripartum acute renal failure, thrombotic microangiopathies, malignant hypertension, scleroderma, renal allograft rejection and glomerulonephritis [1–8]. In experimental models of renal injury intraglomerular fibrin deposition occurs following injection of bacterial lipopolysaccharide (LPS) [9] as well as following accumulation of glomerular antigen–antibody complexes and binding of anti-GBM antibodies to the glomerular basement membrane [2, 11–14]. The generalized Shwartzman reaction induced by two doses of bacterial endotoxin (LPS) given 24 hours apart has been used as a model of endotoxemia, disseminated intravascular coagulation and glomerular capillary thrombosis leading to renal cortical necrosis [9, 10]. The mechanisms responsible for the activation of the coagulation system within the kidney are not yet fully understood [8]. Several different types of cultured cells in vitro have been shown to be capable of producing PCA following stimulation by substances such as LPS, immune complexes, C5a or monokines [15–22]. In particular monocytes are capable of being induced by activated lymphocytes to produce not only tissue factor but also activated Factor VII and a prothrombinase which does not appear to be Factor Xa [16, 20, 21]. Cultured endothelial cells synthesize tissue factor in response to LPS, interleukin I and Tumor Necrosis Factor [15, 22]. Recent studies have shown that glomeruli of rabbits with nephrotoxic nephritis develop high levels of PCA at the time that glomerular fibrin formation occurs [23, 24]. In the present study we have measured and characterized PCA in the kidney in normal and LPS-treated rabbits to determine what the normal distribution of PCA is, and to find out whether PCA increases in vivo under conditions where intravascular fibrin formation occurs in the kidney.

Received for publication July 29, 1985,
and in revised form November 13, 1986

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Methods

Normal renal tissues

Male New Zealand white rabbits weighing between 2 and 2.5 kg were used. Animals were allowed food and water ad libitum before experiments. The animals were anesthetized with pentobarbital 10 to 15 mg/kg intravenously. A midline laparotomy was performed, exposing the abdominal aorta and vena cava, as well as the left kidney. After blunt dissection of the main vessels, the aorta (above the level of the inferior mesenteric artery), and the inferior mesenteric artery were clamped with hemostats. A gauge 14 over the needle teflon catheter (Abbocath, Abbot Hospitals Inc., North Chicago, Illinois, USA) was introduced into the abdominal aorta below the level of the renal arteries in an upstream direction. The inferior vena cava was then cut to allow fluid to escape, and 50 to 150 ml of 0.9% sterile saline solution was infused in order to flush the blood out of both kidneys until they had completely blanched. The pedicle of the right kidney was then clamped and 40 ml of a 0.75% solution of iron oxide in 1% BSA was slowly injected

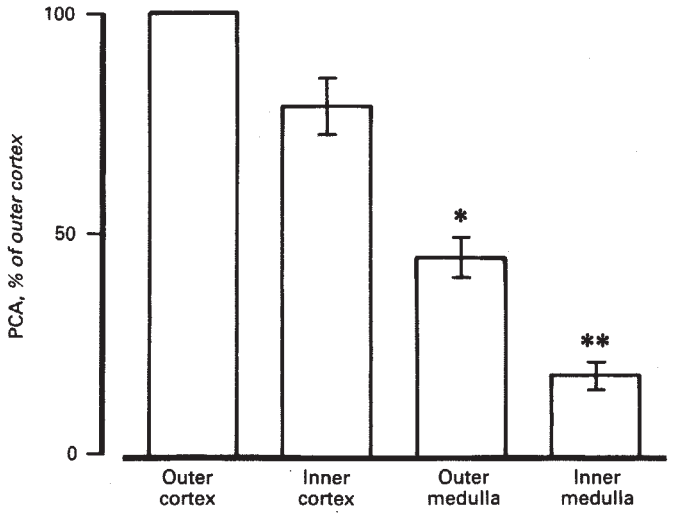

Fig. 1. *Comparison of the amount of procoagulant activity in different regions of the kidney.* PCA was measured as units per μg in 31 different experiments and expressed as percent of the value for outer cortex. The values for inner and outer medulla were significantly different from the value for inner cortex (*$P < 0.05$, **$P < 0.02$).

through the abdominal catheter, turning the left kidney to a greyish color. The animal was sacrificed by pentobarbital (50 mg/kg) and both kidneys were harvested and treated separately. Samples of the right kidney were placed in formal–saline for light microscopy; the rest was macroscopically separated into four different regions: outer cortex, inner cortex, outer medulla, and inner medulla. Each region was diced with a razor blade, suspended in 2 ml of 0.15 M NaCl, sonicated for three minutes, and frozen until assay. The cortex of the left kidney was separated (easily identifiable by the iron particles trapped in the glomeruli), minced and sieved through different sieves: 250 μm copper, 250 μm nylon, 180 μm copper, and 81 μm nylon. Glomeruli were collected from the top of the last screen. They were rinsed with 0.9% saline into a beaker, which was then placed on a magnet until the glomeruli adhered to the base of the beaker (about 1 min). The supernatant (containing the tubular fragments) was poured off. This precedure was repeated two to three times to obtain a clean glomerular preparation (assessed under a light microscope to be more than 95% glomeruli. Fig. 1). Both the glomerular sample as well as the tubular fragment sample were then suspended in saline, sonicated and frozen at −30°C until assay.

Bacterial lipopolysaccharide–treated rabbits

A control and an experimental group consisted of five animals each. The control group received no manipulation, whereas the experimental group was injected with both a preparative and a provocative dose of E. coli LPS (055:B5 Difco, Detroit, Michigan, USA) of 25 μg/kg and 50 μg/kg, respectively, administered 24 hours apart. Rabbits were sacrificed four to six hours after the second injection.

Coagulation assay

The procoagulant activity of the samples was measured employing a one–step coagulation assay using 100 μl volumes of the following reagents: the sample for assay (precentrifuged at 1,000 ×g for one minute to remove any strands of material that might cause the fibrometer to read false fibrin strands) was incubated at 37°C with 25 mM, $CaCl_2$ and pooled, filtered, normal human plasma (anticoagulated with sodium citrate 0.38%). The time taken for a fibrin strand to form was measured using a fibrometer (Fibrosystem BBL, Benton Dickinson & Co., Cockeysville, Maryland, USA). A standard curve was made using progressive dilutions of rabbit brain thromboplastin (Sigma Chemical co., St. Louis, Missouri, USA), and the results, expressed in seconds, plotted against dilution in a log–log plot. This standard curve was linear between 20 seconds and 300 seconds and the slope was such that a twofold *decrease* in clotting time represented approximately a 10-fold *increase* in PCA. Similarly, increasing dilutions of the sonicates from different rabbit kidney regions and structures were plotted, and shown to be parallel to the standard curve. Samples were run in duplicate, and the mean value of both determinations converted into arbitrary units derived from the standard curve as previously described [23]. The values obtained were factored by protein concentration, and expressed as units/μg of protein.

Human plasmas deficient in Factors XII, VII and X (George King BioMedical Inc., Overland Park, Kansas, USA) were used to further characterize glomerular and tubular PCA in a one stage clotting assay as described above. Addition of iron oxide to the coagulation assay did not change the clotting times.

Structural studies

For ultrastructural studies kidney tissue was excised from paraffin blocks. Approximately 1 mm^3 tissue samples were deparaffinized in three changes of xylene with gentle rotation over a twelve hour period. The samples were rehydrated through graded alcohols to 0.1 M cacodylate buffer, pH 7.3, and allowed to fix in cacodylate–buffered 4% glutaraldehyde for one hour at 4°C. Postfixation in cacodylate–buffered 2% OsO_4 for one hour at 4° followed, and after two washes with 0.1 M cacodylate buffer the samples were dehydrated through graded alcohols to two changes of propylene oxide. The samples were infiltrated with increasing concentrations of epon:araldite in propylene oxide for approximately three hours and then embedded in pure epon:araldite and cured overnight in a 65°C oven. Ultrathin sections from these embedded samples were prepared on an AO Ultracut ultramicrotome and stained with uranyl acetate and lead citrate. Ultrastructure was examined on a Phillips 400 transmission electron microscope. Light microscopic analysis was performed from sections stained with hemotoxylin and eosin.

Glomerular culture

Glomeruli were isolated from rabbit renal cortex as described above except that the isolation was performed under sterile conditions with washing steps done with sterile RPMI 1640 containing antibiotics (gentamycin 20 μg/ml and penicillin 100 μ/ml). Glomeruli were then resuspended in the medium at a concentration of 1000 glomeruli per ml. One ml (1000 glomeruli) were placed in each well of a 24 well plate (Costar 3424, 16 mm well diameter, Data Packaging, Cambridge, Massachusetts, USA). LPS (E. coli 0111.4B, Difco) or interleukin-1 (IL-1; Cistron Technologies, Pinebrook, New Jersey, USA) was added to wells to a final concentration of 100, 10, 1, 0.1 μg/ml of LPS and 10 and 1 μ/ml of IL-1. Plates were incubated in a CO_2

humidified incubator at 37°C for 6 hours or 21 hours. At the end of this time the plates were frozen and thawed three times and the procoagulant activity measured by recalcification time as described above. Human renal cortex was obtained from surgical specimens in the operating room. Kidneys removed for carcinoma of the kidney had a normal pole resected and placed in a sterile container on ice. The cortex was removed, minced and the glomeruli sieved through 120 μm nylon sieves. The glomeruli were collected on 80 μm sieves. They were washed thoroughly in RPMI 1640 containing antibiotics (gentamycin 20 μg/ml, penicillin 100 μ/ml) and then handled in the same way as rabbit glomeruli.

Miscellaneous

Iron oxide was prepared according to the method of Cook and Pickering [25]. Briefly, a solution of 20 g potassium nitrate plus 2.6 g sodium hydroxide in 100 ml 95% oxygen–saturated water was mixed with a solution of 9 g ferrous sulfate in 100 ml 95% oxygen–saturated water and brought to boil while constantly stirred. The resulting iron oxide was allowed to precipitate on a magnet for two days, after which it was washed and distilled water by mixture and decantation for five minutes (always with the beaker on the magnet). Finally, the water was discarded, the leftover sludge weighed, and a stock solution prepared using 0.9% saline down to a final concentration of 50 mg/ml. Before infusion, it was diluted to a 0.75% solution in BSA. Copper sieves were obtained from VWR (San Francisco, California, USA) and the nylon screens from Tetko Inc. (Elmsford, New York, USA).

Protein was measured using the Lowry et al method [26].

Statistical significance was tested utilizing Student's *t*-test.

Results

Distribution of PCA in the normal kindey

The PCA measured in various layers of normal kidney is shown in Figure 1. The data are expressed as percent of the outer cortex value to allow comparison of 31 studies done at different times, with different standard curves. It is clear that the most PCA is in the cortex and that least PCA is in the medulla.

To further analyze the distribution of PCA within the cortex, glomeruli and tubules were separated using the iron oxide method (**Methods**). The glomerular and tubular preparations used were greater than 95% free from contamination by tubules and glomeruli respectively (Fig. 2). The PCA content of the glomerular preparation was 21.6 ± 8.8 U/μg protein while that of the tubular preparation was 13.9 ± 2.6 U/μg protein (P = 0.41, N = 9). We conclude that from these data that most of the PCA in the normal kidney is in the cortex where it is distributed to both glomerular and tubular structures.

Distribution of PCA in kidneys of LPS-treated animals

Two groups of five rabbits were compared, one of which had received two doses of LPS 24 hours apart to induce the Shwartzman phenomenon as described in the Methods section. The major histological abnormalities seen in the LPS-treated rabbits are shown in Figure 3. Extensive fibrin formation was seen within glomeruli capillaries, whereas the tubuli and paratubular capillaries and the medulla were relatively spared.

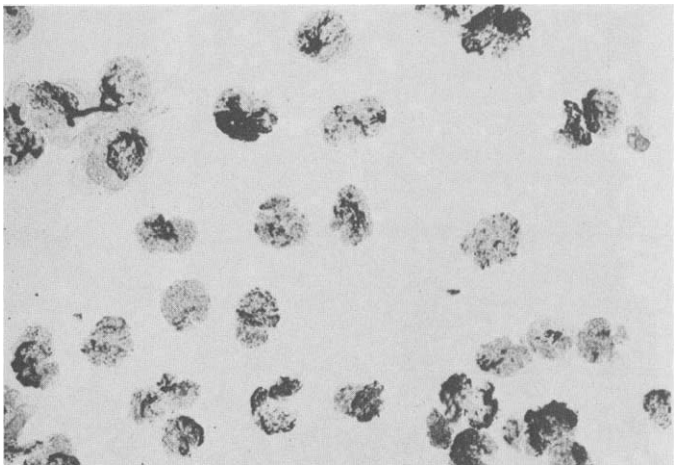

Fig. 2. *Glomerular preparation isolated by the magnetic method using iron oxide.* The black material is iron oxide.

In addition to fibrin and erythrocytes, neutrophils and monocytes were present in glomerular capillaries (Fig. 4). These cells were present in the absence of fibrin and were therefore not merely trapped by fibrin plugs.

The results of measurement of PCA in the different regions of the kidney is shown in Figure 5. An increase in PCA in all layers was observed although the relative distribution between cortex and inner medulla was similar to that seen in normal kidney with there being significantly less PCA in the inner medulla than in the outer cortex ($P < 0.05$).

Glomerular and tubular preparations were also assayed for PCA. Glomeruli from LPS-treated rabbits contained more PCA than did glomeruli from control rabbits (88.8 ± 20.7 vs. 21.6 ± 8.8 units/μg; N = 9). This difference was statistically significant (P = 0.01). The tubular fragment preparations from control and LPS-treated rabbits contained 13.9 ± 2.6 and 44.6 ± 12.7 units/μg, respectively (P = 0.02). The difference between glomeruli and tubular fragments in the control group was not statistically significant (P = 0.41). For the LPS group the glomerular PCA (88.8 ± 20.7 units per μg protein) tended to be higher than tubular PCA (44.6 ± 12.7 units per μg protein), P = 0.07. The glomerular preparation was greater than 95% glomeruli. The tubular preparation was contaminated by glomeruli (2 to 15%) probably because intraglomerular thrombosis prevented ion oxide from embolizing into some glomeruli. From these results we conclude that PCA in LPS-treated rabbits increased markedly, and that more PCA was present in glomeruli than tubules. This increased PCA was associated with fibrin formation in glomeruli.

Characterization of PCA present in normal and LPS-treated kidneys

The type of PCA present in normal and LPS-treated tissues was analyzed by clotting assay using human plasmas deficient in various coagulation factors. The results are shown in Table 1. The clotting times of all preparations were markedly prolonged by the absence of Factor VII but were not prolonged in Hageman factor–deficient plasma. This finding that Factor VII is required for PCA is consistent with the conclusion that

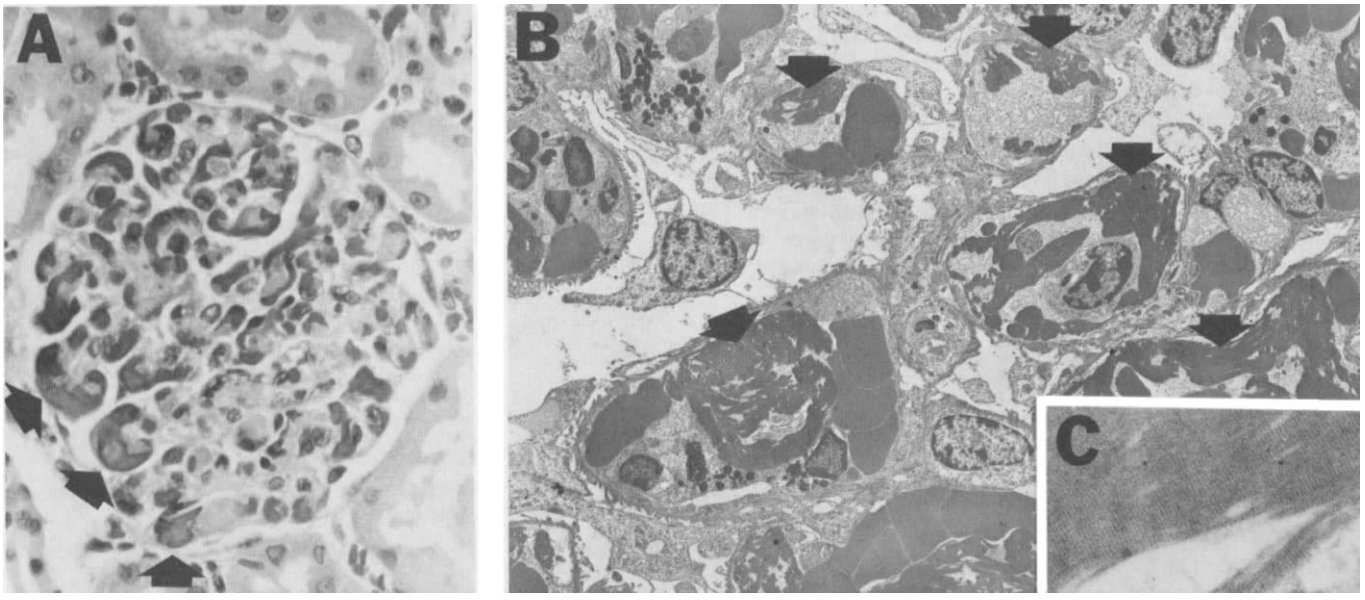

Fig. 3. *The major changes seen in kidneys of LPS-treated rabbits were in glomeruli where there was extensive fibrin formation within glomerular capillaries.* **A.** Light micrograph of section stained with hematoxylin and eosin. The arrows point to fibrin in glomerular capillaries (× 150). **B.** Transmission electron micrograph part of a glomerulus of an LPS-treated rabbit. Arrows point to fibrin within glomerular capillaries (× 790). **C.** Higher magnification shows the typical fibrillar appearance of fibrin (× 17,250).

thromboplastin was the major procoagulant signal in glomeruli from both normal and LPS-treated rabbits.

Incubation of isolated glomeruli with LPS and interleukin-1

Rabbit glomeruli isolated by the magnetic method were incubated with agents which stimulate tissue factor synthesis by cultured endothelial cells. Rabbit glomeruli (1000 per ml) were incubated with LPS (100, 10, 1, 0.1 μg/ml) or interleukin-1 (10, 1 μ/ml) in RPMI 1640 for six hours (3 experiments) or 21 hours (2 experiments). Glomeruli were then freeze–thawed three times and assayed for PCA by clotting assay (**Methods**). No increase in PCA in the presence of LPS or IL-1 was detected in any of the experiments (data not shown). In two experiments sieve–purified human glomeruli were incubated with LPS (10, 1, 0.1 μg/ml) and interleukin-1 (10, 1 μ/ml) in RPMI 1640 for six hours and 21 hours. Glomeruli were freeze–thawed and assayed for PCA. No increase in PCA in the presence of LPS or IL-1 was detected in either experiment (data not shown). We conclude from these data that glomerular cells in situ do not respond to LPS or IL-1 to produce tissue factor under the conditions of these experiments. This is in contrast to experiments carried out with cultured human umbilical–vein, endothelial cells with the same reagents under the same conditions were marked tissue factor production was induced [27].

Discussion

Previous studies have established that normal renal cortex and glomeruli contain tissue factor–like procoagulant activity [28, 29]. The purpose of this study was to examine the distribution of PCA under normal and abnormal conditions in the kidney. The finding that the normal renal medulla contains very small amounts of PCA has also been reported previously [28] and is striking. It might possibly represent a protective mechanism in an area where blood flow is very slow. The normal renal medulla also contains large amounts of fibrinolytic activity [28, 30, 31] which might also function to reduce medullary fibrin formation. Whether this low level of PCA and high level of fibrinolytic activity is related to persistent bleeding into the urinary tract seen under some circumstances is of potential clinical interest.

The Shwartzman reaction is a model for glomerular thrombosis and renal cortical necrosis similar to that seen in septic abortion in man [32]. Many reports have examined the effects of manipulations of the coagulation system on the Shwartzman reaction [reviewed in 9]. It is now well established that anticoagulation (heparin, coumadin, ancrod) protects against both glomerular thrombosis and cortical necrosis whereas preventing fibrinolysis (with E-amino caproic acid) enhances both glomerular fibrin accumulation and cortical necrosis. Plasminogen activators are also protective [33–36]. Vasodilators protect from cortical necrosis but not glomerular thrombosis [9]. Intravenous injection of formed fibrin results in accumulation of fibrin in the glomerulus, but only if fibrinolysis was inhibited [37], and cross circulation experiments have demonstrated accumulation of fibrin in glomeruli of the control rabbits which had been prepared with a single injection of LPS [38]. From these important studies we have the concept that fibrin can form in the circulation, and accumulate on the glomerular filter where, in the presence of vasocontriction, it forms an effective plug preventing blood from reaching cortical structures and thereby causing cortical necrosis. The fact that glomerular fibrinolysis has been shown to be inhibited following intravenous injection of LPS [39] probably as a result of platelet or monocyte/macrophage release of the 50 kilodalton inhibitors of plasminogen activation (PA inhibitor) PAI-1 or PAI-2 [40–44] supports this conclusion. However, accumulation of fibrin

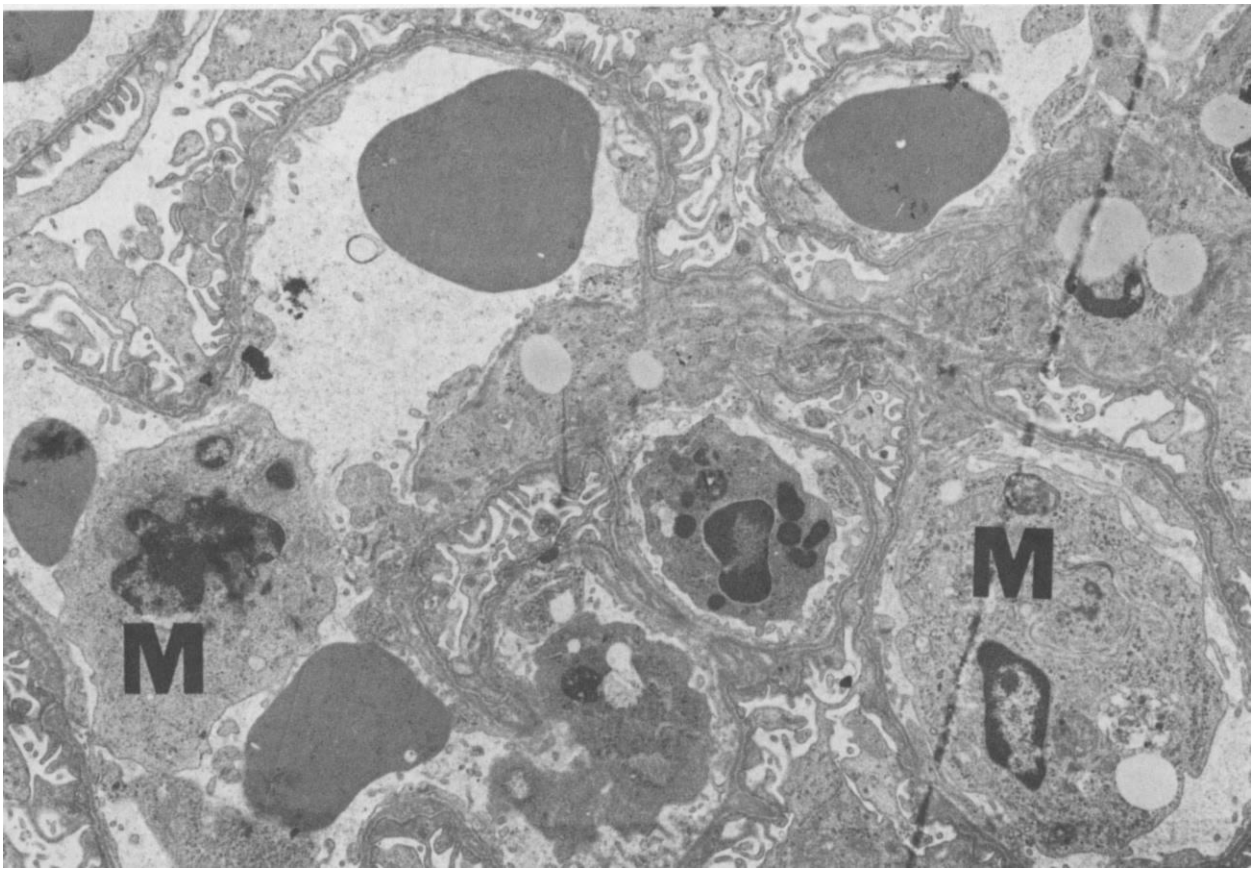

Fig. 4. *Electron micrograph showing part of a glomerulus from an LPS-treated rabbit.* Mononuclear cells (M) are present in glomerular capillaries. No fibrin is present. The diagonal line is a cutting artefact (× 7,400).

which is formed outside the kidney on the glomerular filter cannot be the only mechanism involved because intravascular fibrin formation is also a feature of the *localized* Shwartzman reaction.

To explain these events we need to understand how bacterial lipopolysaccharides cause fibrin formation. There is *no* evidence that the Hageman factor system plays an important role [45, 46]. The fact that leukocyte depletion prevents glomerular thrombosis points to the leukocyte as the key cell [47, 48]. It is now well established that monocytes synthesize tissue factor when incubated with LPS in the presence of serum [49, 50]. The serum factor may be a complement protein [51]. However, the situation is more complex, because monocytes also synthesize Factor VII and a prothrombinase (not Factor X) when incubated with LPS in the presence of lymphocytes [20, 21]. This lymphokine–dependent interaction is HLA-restricted [18–21]. The rabbit hepatic macrophage also produces the prothrombinase molecule when incubated with LPS [52].

A second major potential source of tissue factor is the endothelial cell [15, 53]. Cultured endothelial cells incubated with LPS synthesizes tissue factor in the absence of serum. They also facillitate fibrin formation by promoting coagulating on the cell surface [54]. In addition the monokines Tumor Necrosis Factor and interleukin-1 also cause endothelial cells to synthesize tissue factor [22], and the combination of interleukin 1 and very small amounts of LPS is additive [27]. From these in vitro studies we therefore have a concept of several routes for the synthesis of the procoagulant signal in response to LPS. How does this information fit in with the Shwartzman model?

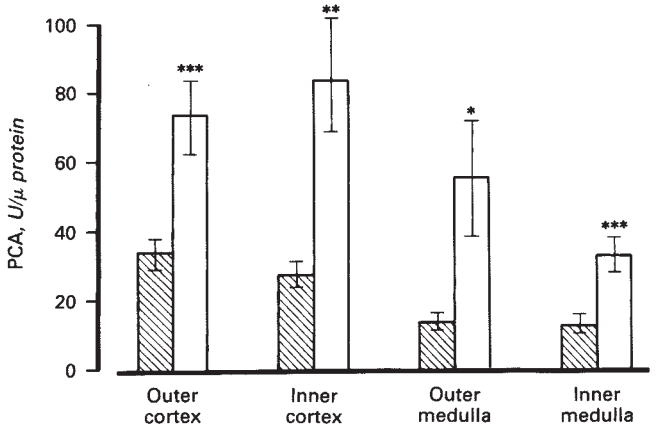

Fig. 5. *Comparison of PCA in different regions of kidney from nomal (▨) and LPS-treated (□) rabbits (N = 5 per group).* The stars indicate the significance of differences between control and LPS-treated animals (*$P < 0.05$, **$P < 0.02$, ***$P < 0.01$). Within the LPS-treated group the value for the inner medulla was significantly lower than that for outer cortex ($P < 0.05$).

Leukocyte depletion studies in the rabbit which prevent *both*

Table 1. Clotting times (sec) measured in different plasmas

Test substance	NHP	XII-D	VII-D	X-D
Normal kidney				
Outer cortex	57.0	61.1	122.3	>300
Inner cortex	66.7	69.8	113.9	>300
Outer medulla	67.6	62.6	197.9	>300
Inner medulla	173.7	192.9	269.0	>300
Thromboplastin	67.5	65.9	255.2	>300
Normal glomeruli				
Preparation A	42.2	51.8	195.0	>300
Preparation B	62.0	69.9	205.0	>300
Thromboplastin	24.3	27.6	131.1	>300
LPS glomeruli				
Preparation C	26.8	25.1	68.6	>300
Preparation D	32.4	ND	80.9	>300
Thromboplastin	22.9	20.8	147.8	297

Representative clotting times from experiments using human deficient plasmas. Abbreviations are: NHP, normal citrated human plasma pool; XII-D, Hageman factor–deficient plasma; VII-D, Factor VII-deficient plasma; X-D, Factor X-deficient plasma; ND, not done. The values given are the mean of duplicate measurements. Each experiment included a rabbit brain thromboplastin control. Normal glomeruli were glomeruli isolated from normal rabbit kidney. LPS glomeruli were glomeruli isolated from kidneys of LPS-treated rabbits. The slope of dilution of rabbit brain thromboplastin is such that a doubling of the clotting time represents approximately a 10-fold decrease in amount of PCA. The dilution curves of glomerular and kidney preparations were parallel to that of the thromboplastin. Note that the absence of Hageman factor did not prolong clotting times whereas the absence of Factor VII did prolong clotting times.

glomerular thrombosis and cortical necrosis have used cytotoxic agents that deplete both neutrophils and mononuclear cells [47, 48]. Although attention has been focussed on the neutrophil [55–57] and some reports have suggested that the neutrophil can synthesize tissue factor in vitro [57, 58] we have not been able to confirm these findings [27]. Although neutrophil preparations do synthesize tissue factor and can be shown to enhance tissue factor production by cultured endothelial cells, these effects are due to contamination of the neutrophil preparation by small numbers of mononuclear cells (< 1%). At this time it therefore appears that mononuclear cells (or macrophages) and/or endothelial cells are the most likely source for the procoagulant signal following intravenous injection of LPS. This concept is supported by studies showing that monocyte preparations from blood, marrow and spleen have increased procoagulant activity following LPS injection in the rabbit [59].

In this report we showed that renal cortical procoagulant activity also increases after two LPS injection in the rabbit and tha this increase appears to be in both glomerular and tubular preparations, although glomeruli showed the higher levels. The procoagulant activity was largely tissue factor–like. Since normal glomeruli contain both mononuclear cells of bone marrow origin and endothelial cells either of these cell types might be responsible without requiring additional help from circulating cells. Indeed, endothelial cells themselves synthesize interleukin-1 when stimulated by LPS [60], and bacterial lipopolysaccharides have been shown to accumulate in the kidney following intravenous injection in the rabbit [61]. However when we incubated isolated glomeruli with LPS we detected no increase in synthesis of tissue factor. We reasoned that the embolized iron might prevent access of LPS to endothelial cells or mesangium so we repeated the experiment in human glomeruli isolated by sieving alone. Again we could detect no increase in tissue factor synthesis. This result could still be artefactual due to poor access of LPS to glomerular cells for some reason. However, it tends to argue against intrinsic glomerular cells being the source of enhanced tissue factor production. An alternative explanation could be that monocytes from the circulation had accumulated in glomerular capillaries or mesangial region. This possibility is supported by ultrastructural studies which showed that monocytes were present in glomerular capillaries even when fibrin was not present. They were therefore not merely trapped by fibrin plugs but appeared to have adhered to glomerular endothelial cells. Recent studies have emphasized the effect of LPS and monokines (IL-1 and TNF) on inducing expression on the endothelial cell surface of both tissue factor and proteins which mediate adhesion of leukocytes [62–64].

From these data we have a concept of mononuclear cells accumulating in the glomerulus to provide a procoagulant signal and possibly promoting a procoagulant signal by intrinsic glomerular cells (endothelial cells or resident mesangial macrophages under the influence of LPS or monokines). This concept fits in with recently published data from models of anti-GBM disease in the rabbit. In one study using a passive model, the increased glomerular tissue factor and fibrin formation could be prevented by leukocyte depletion and partially restored by infusion of peritoneal macrophages into the leukocyte–depleted animals [24]. In our study using an active model, the procoagulant signal corresponded with the appearance of fibrin in Bowman's space and in glomerular capillaries and was associated with the influx of mononuclear cells [23]. Taken together these studies suggest a role for *local* production of fibrin in glomeruli in addition to fibrin forming elsewhere and becoming deposited on the glomerular filter.

Acknowledgments

This work was supported in part by USPHS Grant AM 30673, and a grant–in–aid from the American Heart Association during the tenure of an Established Investigator from the American Heart Association, and with funds contributed in part by the Michigan Branch of the American Heart Association.

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